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Plunkett

Atty. Dkt. No. 016761/0153

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Roy A. BLACK, *et al.*

Appl. No.: 09/244,984

Filing Date: February 4, 1999

Examiner: M. Zeman

Art Unit: 1631

Title: CRYSTALLINE TNF-α-CONVERTING ENZYME AND USES THEREOF

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
Washington, D.C. 29231

Sir:

I, Dauphine S. Torrance Barone, declare as follows:

(1) I received a Bachelor of Science degree in 1980 from the University of California, Riverside. From 1984 to 1987 I was a Research Assistant in the Department of Cell Biology at Calgene, Inc. I have worked at Immunex Corporation as a researcher in the area of Immunology since 1989, and I am currently an Associate Staff Scientist in the Department of Immunobiology at Immunex. I have more than 10 years of experience in analyzing the role of cytokines in animal models of disease, and in this context, I possess particular expertise relating to the measurement of serum TNF-alpha in a mouse endotoxemia model.

(2) I have reviewed an Official Action, with a mailing date of April 25, 2001, which I understand was issued in connection with the above referenced application. From commentary on pages 4 - 6 of the Action, I see that Examiner Zeman alleges that the instant application "does not reasonably provide enablement for designing other associating compounds to native, and full length TACE."

For the reasons elaborated below, I conclude that a person knowledgeable in crystallography, circa 1998, readily could use the claimed methods, in light of the instructions provided, to identify compounds that associate with native, membrane-bound (i.e. 'full-length') TACE.

Using the atomic coordinates supplied by the invention, a research team at the Wyeth division of American Home Products Corporation designed compounds for association with a TACE catalytic domain. I used an *in vivo* mouse model to evaluate the functionality of these compounds, *i.e.*, their ability to associate with a native TACE molecule comprising a catalytic domain.

Because TACE is by far the predominant protease involved in converting TNF-alpha from the membrane-bound precursor form to the soluble mature form, measuring the levels of mature TNF-alpha in serum is a frequently used measure of inhibition of TACE *in vivo*. Furthermore, it is helpful to measure serum TNF-alpha levels under conditions that would normally generate increased levels of serum TNF-alpha, for example, in response to an immunological challenge such as administration of bacterial lipopolysaccharide (LPS), which when administered at high dosages causes lethal endotoxemia (septic shock). Another treatment that generates increased serum levels of TNF-alpha *in vivo* is called Super-antigen-or Anti-CD3-Induced Systemic Inflammation (SAISI).

In these experiments (except for the test of Compound 8, which was tested using SAISI), mice were given an intravenous injection of 40 nanograms of *Escherichia coli* strain 0127:B8 LPS suspended in saline. The LPS was injected 30 minutes after administration of either a compound suspended in a diluent, the compound to be tested for inhibition of production of serum TNF-alpha, or diluent alone as a negative control. The amount of each compound that was administered is shown in the attached Exhibit A. As a positive control, some additional mice were treated in parallel with LPS injected 30 minutes after administration of 25 micrograms of a known TACE inhibitor, Immunex Compound 3 (Black *et al.*, 1997, A metalloproteinase disintegrin that releases tumor necrosis factor-α, *Nature* 385: 729; Immunex Compound 3 is a derivative of Immunex Compound 2, *see* Mohler *et al.*, 1994, Protection against a lethal dose of endotoxin by an inhibitor of tumor necrosis factor processing, *Nature* 370: 218). For the experiment with Compound 8, mice were treated with 5 micrograms of mouse anti-CD3 antibody by footpad injection, either in combination with

Compound 8 suspended in a diluent, or in combination with only the diluent as a negative control. In all cases, serum samples were taken from the mice 1 hour after LPS injection, and serum levels of TNF-alpha were determined by ELISA assays. This protocol is a modification of experimental methods found in the following references: Mohler *et al.*, 1993, Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists, *J Immunol* 151: 1548; and Mohler *et al.*, 1994, Protection against a lethal dose of endotoxin by an inhibitor of tumor necrosis factor processing, *Nature* 370: 218.

The results of these experiments are shown in Exhibit A. Thirteen compounds were tested: Compounds 3 through 6 and 8 through 16. Percent inhibition was determined for each compound by the formula: 100 - (X/Y * 100), where X = serum TNF-alpha levels of LPS/compound-treated mice, and Y = serum TNF-alpha levels of LPS/diluent-treated mice. Therefore, compounds that reduced serum TNF-alpha levels completely (0 picograms per milliliter TNF-alpha) would show 100% inhibition. The inhibition data shown in Exhibit A indicate that eleven of the thirteen compounds effectively associated with the TACE catalytic domain of a native, full-length TACE to provide some degree of inhibition of conversion of TNF-alpha to a soluble form, and eight of the thirteen compounds tested inhibited native TACE by at least 75%. Accordingly, I conclude that the instant application provides sufficient guidance to those in the art to design compounds which associate with native, full-length TACE.

(3) I declare further that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like are made with knowledge that willful false statement and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

October 12, 2001
Date

Dauphine S. Torrance Barone

Serial No.: 09/244,984 Atty. Dkt. No.: 016761/0153

Exhibit A

Inhibition of Serum TNF-alpha Levels Induced by LPS or Anti-CD3 (SAISI)

Compound	Compound Dose in Micrograms (Route)	%Inhibition	
3	100 (IP)	91	
4	100 (IP)	89	
5	50 (IP)	99	
6	100 (IP)	100	
8	100 (PO)	53	SAISI
9	100 (PO)	48	
10	100 (IP)	82	
11	100 (PO)	75	
12	100 (PO)	100	
13	50 (PO)	0	
14	50 (PO)	9	
15	50 (PO)	0	
16	50 (PO)	92	

IP = Intraperitoneal

PO = Per os (orally)

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MESSAGE:

Examiner Zeman:

Enclosed please find the aforementioned R.132 declaration for the above-referenced application.

I look forward to discussing the case with you at 9:30 am on Monday.

Best regards,
Brian McCaslin

If there are any problems with this transmission or if you have not received all of the pages, please call Contact Phone Number 202-945-6044.

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